Incubation Temperature Affects Plasma Insulin-Like Growth Factors in Embryos from Selected Lines of Turkeys[1]

V. L. Christensen,*,[2] J. P. McMurtry,† W. E. Donaldson,* and K. E. Nestor‡

*Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608; †USDA, ARS, Livestock and Poultry Science Institute, Growth Biology Laboratory, Beltsville, Maryland 20705; and ‡Department of Poultry Science, Ohio State University, Ohio Agriculture and Development Center, Wooster, Ohio 44691

ABSTRACT An experiment was conducted to test the hypothesis that incubator temperature may affect circulating insulin-like growth factors (IGF-I and IGF-II). In prior studies, growth of turkey embryos was altered by increasing incubator temperatures. Interestingly, the embryonic growth of a growth-selected line (F) was reduced, whereas embryos from an egg-production-selected line (E) did not alter embryonic growth but altered organogenesis. Growth of the F and E lines was altered experimentally in the current study by increasing incubator temperature from 36.8 to 37.2 C during the last 3 d of incubation. Embryonic blood samples were taken and analyzed for glucose, glucagon, IGF-I, and IGF-II concentrations.

Increased incubator temperature elevated embryonic plasma glucose concentrations of all treatments compared to controls, which was accompanied by increased plasma glucagon concentration only in the E line embryos. Line and treatment interacted to affect IGF-I and IGF-II concentrations of embryo and hatchlings. Line E embryos increased IGF-I in response to the higher temperature, but controls did not; F embryos altered IGF-II in response to treatment, but controls did not. Alterations in IGF-I in E corresponded to growth responses, whereas IGF-II in F corresponded to metabolic responses. We concluded that changes in turkey embryo growth rates to incubator temperature involved changes in IGF-I. Additionally, IGF-II and glucagon are involved in intermediary metabolism during higher temperature exposure.

(*Key words*: insulin-like growth factor, embryo, temperature, turkey, hatching)

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INTRODUCTION

Incubation of turkey embryos at higher than normal temperatures produces opposite effects on survivability of embryos based on whether the genetic lines used are selected for growth or egg production (Christensen et al., 1999a). Elevated temperature slowed growth in growth-selected lines (F) compared to controls. In contrast, elevated incubation temperatures altered only organogenesis in egg-selected lines (E) compared to their controls. The organogenesis response is possibly mediated by insulin-like growth factor (IGF)-I (Bassas et al., 1987; Donath et al., 1998), an anabolic growth factor (Heemskerk et al, 1999). In addition, depletion of glycogen was observed to a greater degree in both randombred lines incubated at elevated temperatures than in the selected lines (Christensen et al., 1999a). Depletion of glycogen may involve glucagon and possibly IGF-II (Spencer et al., 1996; McMurtry et al., 1998b). Thus, elevated incubation temperatures may interact with lines to elicit changes in IGF-I associated with altered organogenesis, whereas slower growth may be associated with IGF-II.

The hypothesis proposed by the current study was that IGF adjust embryonic growth and perhaps metabolism during pipping and hatching of turkey embryos. Our objectives were to measure plasma glucose, glucagon, and IGF-I and -II in embryos incubated at elevated temperatures and to determine whether differences exist in these measures in F and E line embryos compared to their respective randombred controls (RBC2 and RBC1). Glucagon changes were also observed to occur to a greater degree in randombred control line embryos than in selected lines. Recent data suggest that IGF-II may also be involved in intermediary energy metabolism (Spencer et al., 1996; McMurtry et al., 1998b).

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[2]To whom correspondence should be addressed: vern_christensen@ncsu.edu.

Abbreivation Key: E = egg-production-selected line; F = growth-selected line; IGF = insulin-like growth factor; RBC1 = randombred control line from which Line E was selected; RBC2 = randombred control line from which Line F was selected.

MATERIALS AND METHODS

Eggs from four genetic lines (Nestor and Noble, 1995) were incubated together in commercial incubators until the 25th d, which is the beginning of the plateau stage in oxygen consumption (Rahn, 1981). Temperature treatments were applied at that time by increasing incubator temperature (Christensen et al., 1999a). The high-temperature group (high) was placed into machines operated at 37.2 ± 0.1 C, and the control machines (control) were operated at 36.8 ± 0.1 C. Continuous recording thermometers[3] were placed in each machine to verify temperature set points. To account for differences in machines, the same incubation cabinets were not used with the same treatments among trials.

Fertilized eggs were collected from the E line and its randombred control line (RBC1) as well as from F and its randombred control line (RBC2). The breeding stocks were reared by using prior published guidelines, and fertilized eggs were produced as previously reported. Three independent, replicate trials of the study were conducted (Christensen et al., 1993).

At the 25th d of incubation, fertilized eggs with viable embryos were observed by using a candling light to visualize the stage of development. Only the viable embryos were moved to the incubators. At sampling within each of the three trials, three embryos were assigned randomly from among the treatments. The embryo was decapitated, blood was collected into a 10-mL vial containing 10 mg of EDTA, and the vial was placed on ice. Vials were centrifuged (700 × *g*) at 4 C for 10 min. Blood plasma was decanted into storage vials and frozen until analyzed for glucagon, IGF-I (McMurtry et al., 1994), and IGF-II (McMurtry et al., 1998a) by radioimmunoassays. Glucagon was determined as previously reported (McMurtry et al., 1996) Samples were collected immediately prior to pipping (prepip), at internal pipping, at external pipping, at emergence from the shell (hatchling) (a sample size of nine per treatment combination across all three trials). Plasma glucose concentrations were assayed by the glucose oxidase technique described by Donaldson and Christensen (1994).

Data were arranged in a completely random design as a 2 × 2 × 4 factorial arrangement of treatments. As the first factor, the F line embryos were compared to those of RBC2 or the E line embryos were compared to RBC1 embryos. The high-temperature treatment (high) and the control temperature were the two levels of the second factor in the analysis. The third factor was the four stages of incubation at which sampling occurred. Trial was tested as a fixed variable in the analysis, but no trial by treatment interactions were noted so the data were pooled across trials for final analysis. Probability was based on $P \leq 0.05$. Means determined to differ significantly were separated using the least square means procedure of the SAS program for personal computers (SAS Institute, 1989).

[3]Model TempTale H, Sensitech, Beverly, MA 01915.

RESULTS

Blood Plasma Glucose Concentrations

All main effects (line, temperature, and stage) differed significantly for plasma glucose concentrations of the F and control line embryos; however, there were no significant interactions. The control line embryos had higher glucose concentrations than F line (F = 216 mg/dL; RBC2 = 239 mg/dL), and temperature elevated blood glucose concentrations in both lines (high = 233 mg/dL; control = 222 mg/dL). Plasma glucose levels increased significantly at each stage of development (prepip = 184 mg/dL; internal pip = 213 mg/dL; external pip = 236 mg/dL; hatchling = 279 mg/dL). The two-way interactions of line and stage and treatment and stage were significant for plasma glucose of the E and control lines (Table 1 and Figure 1). Higher incubation temperature elevated blood glucose compared to control temperatures in the E line embryos at all stages, but no effects were observed in control line embryos. The higher temperature increased plasma glucose only at internal pipping in both lines.

Plasma Glucagon Concentrations

Only the stage of development affected plasma glucagon levels of F and control line embryos. Plasma glucagon increased at each stage of development (prepip = 61 pg/mL; internal pip = 110 pg/mL; external pip = 213 pg/mL; hatching = 355 pg/mL). Plasma glucagon concentration of E and control line embryos displayed the same line by treatment interaction as was observed for glucose (Table 2). Glucagon concentrations of E line embryos exposed to higher temperatures had elevated glucagon at external pipping and hatching compared to the control line embryos.

Plasma IGF-I and IGF-II Concentrations

The F and control line embryos exhibited no differences in IGF-I concentrations at any time during the experiment. The E and RBC1 embryos displayed significant ($P < 0.0005$) line by treatment by stage interaction (Figure 2). No differences were observed at the prepip and hatchling stages. At prepip, the RBC1 line embryos had elevated IGF-I concentrations in response to higher temperatures, but E line embryos did not. At the hatchling stage, E line embryos had elevated IGF-I concentrations in response to higher temperatures, whereas in the control line embryos IGF-I was suppressed compared to controls.

Plasma concentrations of IGF-II in the F and control line embryos displayed a significant ($P < 0.0005$) line by treatment by stage interaction (Figure 3). Prior to external pipping, IGF-II was suppressed in F line embryos when exposed to higher temperature, but following external pipping it was elevated compared to control line embryos.

TABLE 1. Mean blood plasma glucose concentrations (mg/mL) in turkey embryos (n = 9) selected for egg production (E) compared to randombred controls (RBC1) when incubated at high temperature

		Stage of development				
Genetic line[1]	Treatment[2]	Prepip	Internal pip	External pip	Hatchling	$\bar{x}$
E	High	201	226	243	289	240
	Control	174	206	219	302	225
	$\bar{x}$	187[e]	216[d]	231[cd]	295[a]	
RBC1	High	181	231	248	266	231
	Control	199	214	231	275	230
	$\bar{x}$	190[c]	223[cd]	240[c]	270[b]	
Probabilities						
Line (L)	NS					
Treatment (T)	0.05					
Stage (S)	0.001					
L × T	NS					
L × S	0.03					
T × S	NS					
L × T × S	NS					
$\bar{x}$ ± SEM (n = 144)	232 ± 2					

[a–e]Interaction means with no common superscript differ significantly.

[1]E = line of turkeys selected for increased 180 d egg production; RBC1 = randombred control line from which E was selected.

[2]High = eggs were incubated at 37.2 C at 24 to 28 d of incubation; control = eggs were incubated at 36.8 C at 24 to 28 d of incubation.

Plasma IGF-II concentrations in the E and control line embryos displayed line by treatment and treatment by stage interactions (Table 3). The E line embryos responded to higher temperatures by suppressing IGF-II concentrations across all stages of development compared to controls, but the opposite effect was noted among control line embryos. Embryos of both lines exposed to higher temperatures displayed suppressed levels of IGF-II at internal pipping but elevated levels at external pipping compared to controls (Figure 4).

DISCUSSION

Growth Effects

The evidence from the present study indicates that incubator temperature may be an adequate stimulus to alter

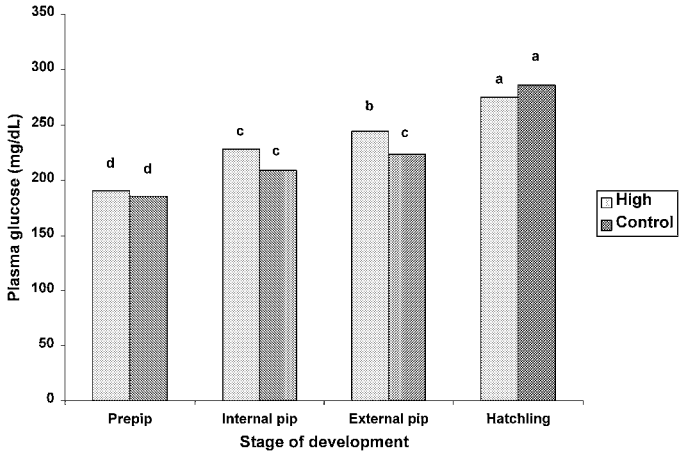

FIGURE 1. Interaction of treatment by stage of development for mean blood glucose concentrations (mg/dL) of F and RBC2 lines of turkey embryos exposed to high (37.2 C) or control (36.8 C) temperatures during stages of hatching. Columns with different superscripts differ significantly ($P \leq 0.05$).

circulating IGF-I and IGF-II in turkey embryos. It was also shown that turkey line interacted with incubation temperatures to affect IGF-I and IGF-II. Exposure of turkey embryos to higher temperatures at the plateau stage in oxygen consumption, at 25 to 26 d of incubation in turkeys, was a far more potent stimulus for altering growth and carbohydrate metabolism of embryos from different lines (Christensen et al., 1999a) than was ambient oxygen tension in prior studies (Christensen et al., 1999b). IGF-I is an anabolic growth factor that induces amino acid uptake and protein synthesis and enhances glucose uptake (Heemskerk et al., 1999). Because of growth differences observed in the prior study (Christensen et al., 1999a), the observation of IGF changes suggests a relationship between embryonic growth rate and plasma IGF in developing turkey embryos. In support of this relationship is the previous report that plasma IGF1 concentrations are depressed in turkey embryos incubated under shell-less conditions (McMurtry et al., 1990; Robcis et al., 1991; McMurtry et al., 1996). In this situation embryonic growth and development are significantly impaired. This result also supports earlier reports that IGF-I may be involved in embryonic growth in other species (Bassas et al., 1987; Donath et al., 1998). In the current study, E embryos that responded to increases in incubation temperature by maintaining growth (Christensen et al., 1999a) responded by increasing IGF-I levels in the hatchling. Conversely, IGF-I levels were unchanged in F embryos that lost 10% of their BW in response to high incubation temperatures. These data suggest that external factors such as incubation temperature are important determinants of IGF production and growth in turkey embryos. This report is the first to show that incubator or environmental temperature influences IGF-I or II plasma concentrations in avian embryos.

IGF are involved in organogenesis and tissue repair (Bassas et al., 1987; DePablo et al., 1991; Stewart and

TABLE 2. Mean blood plasma glucagon concentrations (pg/mL) in turkey embryos (n = 9) selected for egg production (E) compared to randombred controls (RBC1) when incubated at high temperature

		Stage of development				
Genetic line[1]	Treatment[2]	Prepip	Internal pip	External pip	Hatchling	$\bar{x}$
E	High	128	114	286	452	245
	Control	77	137	300	527	260
	$\bar{x}$	102[d]	125[d]	294[bc]	489[a]	252[a]
RBC1	High	85	98	226	295	176
	Control	81	96	249	336	191
	$\bar{x}$	83[d]	97[d]	237[c]	316[b]	184[b]
Probabilities						
Line (L)		0.0003				
Treatment (T)		NS				
Stage (S)		0.0001				
L × T		NS				
L × S		0.01				
T × S		NS				
L × T × S		NS				
$\bar{x}$ ± SEM (n = 144)		219 ± 9				

[a–d]Interaction means with no common superscript differ significantly.

[1]E = line of turkeys selected for increased 180-d egg production; RBC1 = randombred control line from which E was selected.

[2]High = eggs were incubated at 37.2 C from 24 to 28 d of incubation; control = eggs were incubated at 36.8 C from 24 to 28 d of incubation.

Rotwein, 1996; McMurtry et al., 1997; Donath et al., 1998; Kocamis et al., 1998). The higher temperature consistently increased IGF-I and IGF-II in E and RBC1 embryos that experienced different organogenesis at increased temperature. This variation suggests there may be differences in Type 1 IGF receptor activity in various organs between the two lines. Trouten-Radford et al. (1991) have shown that the number of IGF receptors in embryo breast muscle of slow and fast growing chick strains were not different, but receptor densities were greater in the fast growing strain. A similar situation may exist in the organs and muscle of the lines of turkeys used in this study; however, confirmation of this hypothesis awaits further investigation.

Energy Metabolism Effects

The IGF function to regulate processing of metabolic nutrients. IGF-I is an anabolic growth factor that induces amino acid uptake and protein synthesis and enhances glucose uptake (Heemskerk et al., 1999; Herbert and Berkel, 1999). In a recent study, McMurtry et al. (1998b) injected chicken and human IGF-II to determine endocrine and metabolic effects on 5-wk-old broiler chickens. They observed significant depressions in plasma insulin, growth hormone, thyroxine, and triiodothyronine concentrations. Additionally, glucagon, uric acid, calcium, and plasma free fatty acids were elevated, suggesting a relationship between IGF-II and metabolism. There is

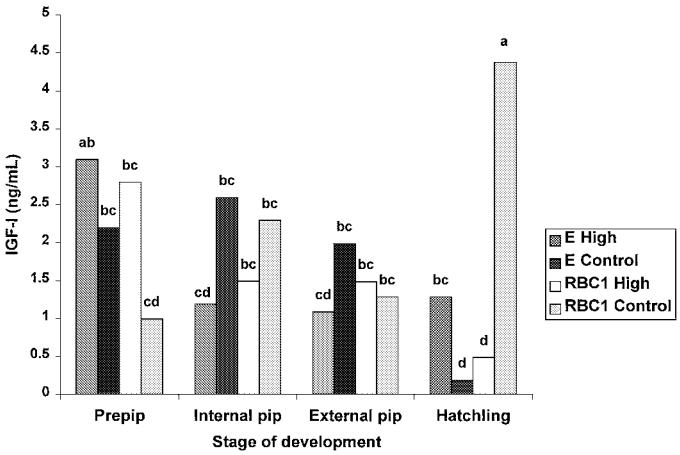

FIGURE 2. Interaction of line by treatment by stage of development for mean insulin-like growth factor-I (IGF-I) concentrations (ng/mL) of E and RBC1 line embryos exposed to high (37.2 C) or control (36.8 C) temperatures during stages of hatching. Columns with different superscripts differ significantly ($P \leq 0.05$).

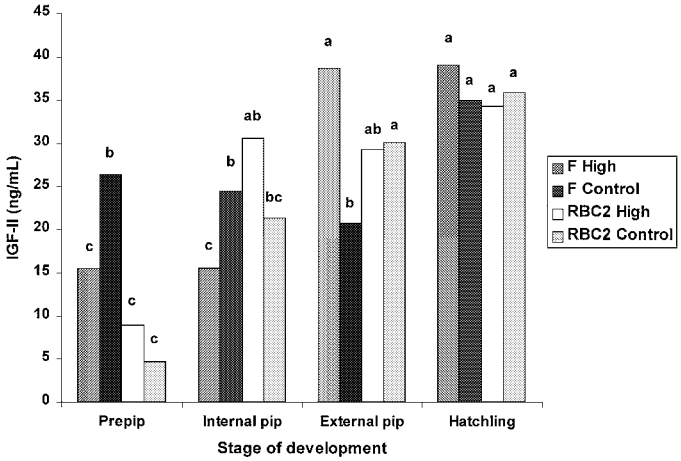

FIGURE 3. Interaction of line by treatment by stage of development for mean insulin-like growth factor-II (IGF-II) concentrations (ng/mL) of F and RBC2 line embryos exposed to high (37.2 C) or control (36.8 C) temperatures during stages of hatching. Columns with different superscripts differ significantly ($P \leq 0.05$).

TABLE 3. Mean blood plasma insulin-like growth factor-II (IGF-II) concentrations (ng/mL) in turkey embryos (n = 9) selected for egg production (E) compared to randombred controls (RBC1) when incubated at high temperature

		Stage of development				
Genetic line[1]	Treatment[2]	Prepip	Internal pip	External pip	Hatchling	$\bar{x}$
E	High	14.0	17.1	31.4	28.5	22.8[ab]
	Control	21.8	25.4	31.9	34.0	28.3[a]
	$\bar{x}$	17.9[b]	21.2[b]	31.7[a]	31.3[a]	
RBC1	High	13.7	13.2	45.5	32.6	26.2[ab]
	Control	2.8	26.3	18.8	36.1	20.9[b]
	$\bar{x}$	8.3[c]	19.8[b]	32.2[a]	34.3[a]	
Probabilities						
Line (L)		NS				
Treatment (T)		NS				
Stage (S)		0.0001				
L × T		0.05				
L × S		0.04				
T × S		NS				
L × T × S		NS				
$\bar{x}$ ± SEM (n = 144)		24.6 ± 3.3				

[a–c]Interaction means with no common superscript differ significantly.

[1]E = line of turkeys selected for increased 180-d egg production; RBC1 = randombred control line from which E was selected.

[2]High = eggs were incubated at 37.2 C at 24 to 28 d of incubation; control = eggs were incubated at 36.8 C at 24 to 28 d of incubation.

some evidence that IGF-II is involved in some aspect of intermediary metabolism, as the administration of exogenous IGF-II to broiler chickens increased the size of the abdominal fat pad (Spencer et al., 1996). In the current study, the altered IGF-I and IGF-II concentrations changed as plasma glucose concentrations increased in response to hatching and temperature. These changes were, however, affected by line differences. Plasma glucagon increased when expected as plasma glucose increased during hatching in the comparison of E and control lines. Plasma glucagon was elevated at the time that E line embryos increased plasma glucose concentrations. However, plasma glucose concentrations increased in the comparisons of treated F and control lines without an increase in plasma glucagon, which is more difficult to understand. Plasma IGF-II concentrations were, however, increased in the high-temperature treatment at the same time as plasma glucose was elevated in F embryos.

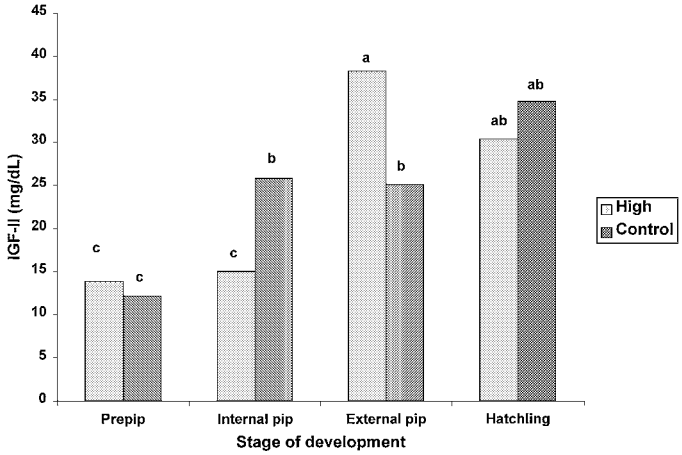

FIGURE 4. Interaction of treatment by stage of development for mean insulin-like growth factor-II (IGF-II) (ng/mL) of F and RBC2 line turkey embryos exposed to high (37.2 C) or control (36.8 C) temperatures during stages of hatching. Columns with different superscripts differ significantly ($P \leq 0.05$).

Less is known about IGF-II than IGF--I in the developing avian embryo. Throughout incubation, IGF--II concentrations are greater than IGF-I in the turkey embryo (McMurtry and Brocht, 1997; McMurtry et al., 1998a). The same observation was made in the current study. In the turkey, plasma IGF-II levels increase dramatically between d 16 and 18 d of incubation, peak just prior to hatching (Day 26), followed by a precipitous decline at hatching (McMurtry et al., 1998a). The elevated levels of IGF-II compared to IGF-I during embryonic development in the current study suggests strongly that it is important to embryogenesis, as IGF-II is considered to be important to mammalian fetal development (Stewart and Rotwein, 1996). Because IGF-II was associated with changes in glucose in the current study, it warrants further investigation. The role of IGF-II in the bird is largely unknown (McMurtry et al., 1997), which is confounded by the fact that avian species do not possess the classic Type 2 receptor (Yang et al., 1991).

Data in the current study suggest physiological roles for IGF-I and IGF-II in turkey embryos. Roles for IGF-I in organogenesis of egg-production-selected embryos were suggested, whereas roles for IGF-II in intermediary energy metabolism were suggested for growth-line-selected embryos.

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